studies of the peptide in a solution of small unilamellar vesicles were conducted and showed that the increase in helical content is also present in the context of close proximity to a lipid membrane. To confirm, single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated state and in the PKCα-phosphorylated state, in order to gauge the distance between two native cysteines in the peptide. Phosphorylation yielded a reduced distance between these cysteines, indicative of a shift to more compressed secondary structure, that is, coil to helix.

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Characterization of a "Hotspot" in the AMPA Receptor Activation Pathway

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Ionotropic glutamate receptors (iGluRs) facilitate the bulk of synaptic excitation in the mammalian central nervous system. Structures of full-length, AMPA-type iGluRs (AMPARs) have recently been reported in conformations thought to represent resting, pre-open, and desensitized states. However, it is uncertain what molecular interactions determine whether an agonist-bound AMPAR will favor channel opening or desensitization. We previously described how the activation of kainate-type iGluRs (KARs) is dependent upon occupancy, by sodium, of an electronegative pocket in the ligand-binding domain (LBD). Subsequently, we asked to what extent this pocket, conserved amongst iGluR subfamilies, regulates AMPAR activation. To investigate this subject we utilized electrophysiological (outside-out patch) recordings from iGluR subunits transiently expressed in HEK 293 cells, as well as molecular dynamics (MD) simulations. Unlike the KAR subunit GluK2, receptors comprised of the AMPAR subunit GluA2 did not require occupancy of the pocket by a positive charge to activate. Interestingly, a lithium ion has been detected in the pocket of recent crystal structures of the GluA2 LBD. The effect of lithium in the external recording solution was to dramatically slow the desensitization kinetics of GluA2. MD simulations supported an increased affinity of the site for lithium versus sodium, and predicted that lithium binding holds subunits closer together. Through disrupting an inter-subunit electrostatic bridge adjacent to the "cation" pocket, the effect of lithium was greatly attenuated. In fact, the removal of key charges at this interface produced receptors barely capable of activation, although the functional deficit was rescued by the modulator cyclothiazide or co-expression with auxiliary subunits. We propose that when electrostatic interactions at the apex of the LBD are stabilized, AMPARs are primed for activation, whereas the disruption of these interactions directs receptors to desensitized states upon agonist binding.

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Dynamics of the Cytoplasmic Region of an AMPA-Subtype Glutamate Receptor Revealed by State Dependent FRET

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AMPA receptors (AMPARs) are glutamate-gated ion channels, which mediate fast excitatory neurotransmission in the central nervous system. Extensive crystallographic studies of the extracellular domains and, more recently, crystal structures and single particle EM reconstructions of full-length receptors have set the framework for further investigations of receptor conformational dynamics, gating mechanism and regulation. However, intracellular regions are either truncated or not resolved in these structures. Further, the conformational transitions of the intracellular domains during receptor gating have not been investigated.

In present study, we have explored single and double fusions of cyan and yellow variants of green fluorescent protein (CFP and YFP, respectively) at intracellular sites of AMPAR to enable measurement of conformational changes using Fluorescence Resonance Energy Transfer (FRET) in live cells. The fluorescent fusions retain wild-type receptor expression and kinetic properties. Fluorescence Lifetime Imaging (FLIM) showed ligand-dependent FRET efficiency. Conformational rearrangements accompanying receptor function were measured using a Patch Clamp Fluorometry (PCF) setup on live HEK 293 cells in real time. Our results suggest that FRET efficiency is dependent on the functional state of the receptor and allosteric modulation by Cyclothiazide, an AMPA receptor desensitisation blocker. Thus the intracellular sites undergo conformational rearrangements during receptor function.

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Partial Agonist Binding Reveals a Unique Arrangement of AMPA LBDs

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Ionotropic glutamate receptors (iGluRs) are large tetrameric membrane proteins that transduce the chemical signal from neurotransmitters into membrane depolarization at synapses in the brain. The conformational transition induced by the association of glutamate molecules to the ligand-binding domains (LBDs) of these receptors provides the free energy that drives the opening of the transmembrane ion channel. Here, we describe the crystal structure of a GluA2 LBD tetramer in presence of the partial agonist 5-fluorowillardiine (FW) (FW sLBDs). Validation of the structure by a battery of engineered metal bridges showed that this LBD configuration corresponds to an intermediate state of receptor activation distinct from the previously publish closed-angle (CA) structure. GluA2 activation therefore, involves a combination of both intra- and inter-LBD dimer conformational transitions. The presented results provide new quantitative data supporting the idea of a dynamic LBD during activation in the context of a tetramer.

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Structural Mechanism of Glutamate Receptor Activation and Desensitization

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Ionotropic glutamate receptors are ligand-gated ion channels that mediate excitatory synaptic transmission in the vertebrate brain. To gain a better understanding of how structural changes gate ion flux across the membrane, we trapped rat AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate receptor subtypes in their major functional states and analysed the resulting structures using cryo-electron microscopy. We show that transition to the active state involves a 'corkscrew' motion of the receptor assembly, driven by closure of the ligand-binding domain. Desensitization is accompanied by disruption of the amino-terminal domain tetramer in AMPA, but not kainate, receptors with a two-fold to four-fold symmetry transition in the ligand-binding domains in both subtypes. The 7.6 Å structure of a desensitized kainate receptor shows how these changes accommodate channel closing. These findings integrate previous physiological, biochemical and structural analyses of glutamate receptors and provide a molecular explanation for key steps in receptor gating.

1442-Pos Board B393

Long Timescale Simulations of Ligand Binding in Glutamate Receptors

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Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels that mediate the majority of fast excitatory transmissions in the central nervous system. They transduce chemical information upon agonist binding into electrical information at synapses. In this study, we present long timescale simulations of both ligand binding association and dissociation events. Novel intermediate states and metastable interactions are identified using potential of mean force (PMF) calculations. Kinetics are inferred using a Markov state model (MSM).

1443-Pos Board B394

Can Activation and Desensitization Properties of iGluRs Be Predicted and Understood by Studying the LBD Dimer Dynamics?

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Ionotropic glutamate receptors (iGluRs) are vital for the function of our central nervous system (CNS), e.g. in learning and memory formation, and thus implicated in many CNS disorders. The tetrameric iGluRs contain a glutamate-gated cation channel with the extracellular ligand binding domains (LBD) forming a dimer of dimers. Subsequent to channel opening,